IN THE CLAIMS

Please cancel claim 14 without prejudice or disclaimer.

A complete listing of all claims is provided herewith in **Attachment C**. It will be noted that claims 1, 2, 3, 6, 11 and 13 are currently amended; claims 15 and 16 have been added; claims 7, 8, 9, 10 and 12 have been withdrawn; and claim 14 has been canceled.

no clean copy of the claims

REMARKS

Claims 1-6, 11, 13, 15 and 16 stand pending in the present application. By this Amendment, Applicants have amended claims 1, 2, 3, 6, 11, and 13, and added new claims 15 and 16. Applicants respectfully submit that the present application is in condition for allowance based on the discussion which follows.

The specification was objected to as being vague for using the term "biologically active" and for using the term "similar" in conjunction with the term "biologically active". In addition, the Examiner noted that it was unclear how similar the activity should be to be within the term "metalloprotease activity". Further, the Examiner asserts that the term "metalloprotease activity" itself is broad because a large number of metalloproteases.

Contrary to the Examiner's allegation, the definition of the term "biologically active", "similar" activity and a "metalloprotease activity" are not vague and, moreover, one of ordinary skill in the art based on the entire disclosure would know precisely to which these terms pertain.

With regard to the term "metalloprotease activity", Applicants respectfully note that metalloproteases are enzymes having catalytic activity which involves a metallic

ion. Accordingly, the one skilled in the art can readily identify an enzymatic activity as being a metalloprotease activity by using a chelatant agent such as EDTA (see, e.g., Appendix A, extract of Roche Molecular Biochemicals, The Complete Guide for Protease Inhibition that describes EDTA Na_2 as a specific inhibitor of metalloproteases).

Furthermore, it can be drawn from the sequence SEQ ID NO: 4 that NEP2 contains a "HEXXH" motif (positions 2 to 6 of SEQ ID NO: 4 which is a characteristic signature of zinc containing metalloproteases (see the instant specification, page 3, lines 19-26).

Therefore the one skilled in the art would recognize that NEP2 is a metalloprotease, more specifically a "zincin", i.e., a zinc containing metalloprotease.

To further evidence that one skilled in the art would readily recognize that NEP2 is a zincin, Applicants have attached an article from the inventors (Rose et al) that further supports that the claimed NEP2 is a zincin, and more specifically a member of the M13 subfamily of metalloproteases (Appendix B, Cell-specific activity of neprilysin 2 isoforms and enzymic specificity compared with neprilysin, Biochem J., 2002, hereinafter "Rose et al").

As shown by Rose et al, NEP2 activity is completely abolished by 1 mM EDTA (see Rose et al, the legend of Table 2, page 701), a result that assigns NEP2 to the metalloprotease family. NEP2 is further inhibited by phosphoramidon, an inhibitor specific for the zincin family of metalloproteases (Rose et al, Table 2).

Therefore, contrary to the Examiner's objection, the term "metalloprotease activity" would be understood by one of ordinary skill in the art.

With regard to the term "similar", in context with the complete present disclosure, and in particular, the whole sentence, the term "similar" has to be understood as referring to properties identical or similar to that of NEP2. In any event, said activity similar to that of NEP2 has to be a metalloprotease activity.

Therefore, Applicants respectfully submit that the term "similar" does not render the definition indefinite.

In view of the foregoing discussion, Applicants respectfully request that the Examiner withdraw the aforementioned rejection to the specification.

The specification was also objected to for incorrectly writing the abbreviation of the term "sequence identification number". By this Amendment, Applications have amended the specification by correctly referring to SEQ ID No. 4 as SEQ ID NO: 4 thereby obviating the Examiner's objection to the specification.

Further, with regard to the specification, the Examiner has requested that Applicants check the specification for possible errors. By this Amendment, Applicants have amended portions of the specification to be more consistent with conventional U.S. patent form and more conventional technical nomenclature.

Claims 1-6, 11, 13 and 14 were rejected under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph. The Examiner alleges that the claimed invention lacks patentable utility. The Examiner notes that the NEP2 protein described by the sequence identifier SEQ ID NO: 4 is identified as a metalloprotease in view of the motif HEITH, responsible for binding the zinc, and the sequence homology with known metalloproteases.

The Examiner further asserts that "annotation of function on the basis of homology to the protein with a well established biologic function is in many cases erroneous". The Examiner cites Seffernick et al to support this assertion.

Applicants respectfully traverse the Examiner's latter assertion. Seffernick et al disclose the identification of two proteins with 98% sequence identity. However, Seffernick et al clearly show that these 98% sequence identical proteins have different activities. Moreover, the authors of Seffernick et al appear to be astonished by their own observations, as expressed in the first two paragraphs of the discussion where they state:

> "The present finding that proteins with > 98% sequence identity catalyze different reactions in different metabolic pathways is highly exceptional",

and

> "while it is surprising that enzymes with such high sequences identify catalyze different reactions . . ." (Seffernick et al, emphasis added).

Applicants respectfully submit that the Examiner has misinterpreted the teaching of Seffernick et al and drawn a general rule from a situation that is described by Seffernick et al themselves as exceptional.

Furthermore, the utility of the claimed NEP2 is further supported by Rose et al who show that NEP2 activity can be assessed using for instance a fluorogenic substrate Succinyl-Ala-Ala-Phe-Amidomethyl-Coumarin (Suc-AAF-AMC, see Rose et al, page 698, right column, section "Partial purification of NEP2(s) and NEP2(m) activities" and Table 1, page 700). NEP2 further hydrolyses bioactive oligopeptides, such as enkephalin, substance P, bradykinin or angiotensin I (see, e.g., Rose et al, Tables 3, 5 and 6). that is the prove!

Based on the foregoing, Applicants respectfully submit that the utility of the claimed NEP2 is supported by the properties disclosed in the present application, i.e., the HEXXH motif of zinc, metalloproteases and the homology between NEP2 and ECE/NEP/Kell enzymes, together with the results from the inventors shown in Rose et al.

With regard specifically to the Examiner rejecting claim 6 under 35 U.S.C. § 101 as directed to the product of nature, by this Amendment, Applicants have amended claim 6 by adding the term "isolated".

In view of the foregoing discussion, Applicants respectfully request that the rejection to claims 1-6, 11, 13 and 14 under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph, be withdrawn.

Claims 1-6, 11, 13 and 14 were rejected under 35 U.S.C. § 112, second paragraph. The Examiner rejected the claims as being indefinite which relate to:

1) a sequence derived from the sequences of SEQ ID NO: 4 or 3,

2) a sequence homologous to the sequences of SEQ ID NO: 4 or 3,

3) a biologically active fragment of SEQ ID NO: 4 (emphasis added).

By this Amendment, Applicants have amended claim 1 to more clearly recite what Applicants believe to be the invention and to be more consistent with conventional U.S. claim form, thereby obviating the rejection to the claims under 35 U.S.C. § 112, second paragraph. Support for the amendment to claim 1 can be found in the specification as filed on page 2, lines 31-36. Further, Applicants have amended claim 2 with support in the specification on page 4, lines 16-21.

With regard to claim 3, Applicants submit that claim 3 is drawn to a probe that hybridizes specifically with a nucleotide sequence according to claim 2 and that the probe has a nucleotide sequence selected from the group consisting of the sequences SEQ ID NO: 5 to SEQ ID NO: 27. Accordingly, Applicants respectfully submit that claim 3 cannot be indefinite. However, in an effort to more clearly define Applicants' invention, Applicants have amended claim 3 by deleting the term "which hybridizes specifically with a nucleotide sequence as claimed in claim 2, said probe".

With regard to claim 6, Applicants respectfully submit that one of ordinary skill in the art would readily know what is included in the claimed monoclonal or polyclonal antibodies, fragments thereof, chimeric antibodies or immunoconjugates that are specific for a polypeptide of the invention.

Christine

With regard to claims 11, 13 and 14, Applicants have amended these claims to include all the essential steps and to be more consistent with conventional U.S. method claim form thereby obviating the rejection to these claims.

Based on the foregoing discussion, Applicants respectfully request that the rejection to claims 1-6, 11, 13 and 14 under 35 U.S.C. § 112, second paragraph, be withdrawn.

Claims 1-6, 11, 13 and 14 were rejected under 35 U.S.C. § 112, first paragraph. The Examiner notes that SEQ ID NO: 3 is 327 nucleotides long and encodes SEQ ID NO: 4 which consists of 116 amino acids. The Examiner thus rejects the pending claims in view of this discrepancy.

Contrary to the Examiner's assertion, claims 1-6, 11, 13 and 14 are fully supported by the specification as filed. SEQ ID NO: 3 is disclosed as a 327 nucleotide long sequence which encodes a partial amino acid sequence of NEP2 in humans (specification, page 3, lines 36-38). Further, SEQ ID NO: 4 is a partial amino acid sequence of NEP2 in humans (specification, page 2, lines 5-6). Since SEQ ID NO: 3 encodes only a portion of NEP2, and not necessarily the entire NEP2, let alone encoding the complete sequence of SEQ ID NO: 4, it is not inconsistent that SEQ ID NO: 3 has only 327 nucleotides rather than 348 nucleotides.

Furthermore, based on Examples 3 and 4 disclosed in the present specification, Applicants have fully demonstrated and have provided support for the claimed subject matter recited in claims 1-6, 11, 13 and new claims 15 and 16. For example, Example 3 is drawn to demonstrating the homology between human NEP2 and rat NEP2 whereas Example 4 is drawn to demonstrating a correlation between human NEP2 protein sequence and a corresponding rat polypeptide sequence. (See specification, Example 3, pages 12-13 and Example 4, page 13.) Therefore, Examples 3 and 4 fully enabled and support the claims which are drawn to an isolated polypeptide of SEQ ID NO: 4 (e.g., claim 1 (currently amended)) and an isolated nucleic acid comprising a nucleotide sequence SEQ ID NO: 3 (e.g., claim 2 (currently amended)). Despite there being a discrepancy in sequence length corresponding between SEQ ID NO: 3 (DNA sequence) and SEQ ID NO: 4 (amino acid sequence to which SEQ ID NO: 3 encodes), nevertheless the specification clearly supports the claimed subject matter drawn to the nucleotide sequence and amino acid sequence. Since the specification clearly demonstrates that SEQ ID NO: 3 is a partial sequence for NEP2 in which SEQ ID NO: 4

is a partial amino sequence thereof, the specification supports SEQ ID NO: 3 being itself a partial sequence encoding SEQ ID NO: 4. In view of the foregoing, Applicants respectfully submit that with regard to SEQ ID NO: 3 and SEQ ID NO: 4, the claims are fully supported and enabled by the specification as filed in accordance with 35 U.S.C. § 112, first paragraph.

In view of the foregoing discussion, Applicants respectfully request that the Examiner withdraw the rejection to claims 1-6, 11, 13 and 14 under 35 U.S.C. § 112, second paragraph for the alleged discrepancy between SEQ ID NO: 3 and SEQ ID NO: 4.

With regard to the rejection of claims 1, 6, 11, 13 and 14 as being directed to a large species of polypeptides that comprise SEQ ID NO: 4, as well as claims 2-5 for reciting DNA molecules comprising SEQ ID NO: 3, Applicants respectfully submit that the aforementioned amendment to the claims obviate the rejection under 35 U.S.C. § 112, first paragraph.

In addition, claims 1, 6, 11, 13 and 14 were rejected under 35 U.S.C. § 112, first paragraph, where the Examiner notes that the specification does provide enablement for the polypeptide identified by SEQ ID NO: 4, but that the specification does not provide enablement for all peptides that comprise:

a) SEQ ID NO: 4,

b) a sequence derived from SEQ ID NO: 4,

c) a sequence homologous to SEQ ID NO: 4, and

d) a biologically active fragment of SEQ ID NO: 4.

Specifically, the Examiner asserts that the specification does not enable any person skilled in the art to which it pertains or to which it is most nearly connected, to make the invention commensurate in scope with the claims. Further, the Examiner asserts that the genus of polypeptides listed under a) – d) is a large and variable genus encompassing the species that do not have the desired functionality.

Contrary to the Examiner's assertion, claims 1, 6, 11 and 13, as currently amended, and newly added claims 15 and 16 recite subject matter fully supported and enabled in the specification as filed with regard to SEQ ID NO: 4. Furthermore, based on the discussion above with regard to the 35 U.S.C. § 112, first paragraph and second paragraph, rejection to the claims, Applicants respectfully submit that the claims are fully supported by the specification as filed to enable one of ordinary skill in the art to practice the invention claimed.

Claims 1 and 2 were rejected under 35 U.S.C. § 102(b) as being anticipated by Shipp et al.

The Examiner alleges that the subject matter of claims 1 and 2 would be anticipated by Shipp et al. The Examiner asserts in particular that the disclosed CALLA antigen would have 70.5% sequence identity with amino acids 1-114 of SEQ ID NO: 4.

As an initial point, contrary to the Examiner's assertion, the sequence alignment provided by the Examiner indicates 76 matching residues on 114, which correspond to only 66.7% sequence identity.

Further, by this Amendment, Applicants have amended the claims to recite that the derived or homologous sequence have at least 75% identity with SEQ ID NO: 3 or SEQ ID NO: 4, thereby make the Examiner's assertion moot.

Claim 6 was rejected under 35 U.S.C. § 102(b) as being anticipated by both Ritz et al and Shipp et al.

As an initial point, as stated by Ritz et al, antisera with CALLA specifically identify a single glycoprotein with a molecular weight of 95-100.00 (see Ritz et al, page 584, right column, second paragraph, and Figure 1, lane g), i.e., the CALLA protein. Thus Ritz et al do not teach the disclosed antisera recognize or even could recognize another protein, such as NEP2.

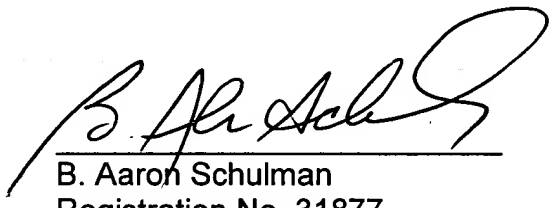
Furthermore, the prior art rejection to claim 6 is now moot over the claim amendment to claim 6. Claim 6 (currently amended) is directed to an antibody specific for NEP2 or for a derived or homologous protein that have at least 75% sequence identity therewith. The CALLA protein does not constitute a protein having at least 75% sequence identity with NEP2.

Furthermore, Applicants respectfully submit that Shipp et al and Ritz et al fail to provide an enabling disclosure to anticipate the claimed invention. Neither Shipp et al nor Ritz et al address the same question as the invention, namely cloning of a novel metalloprotease. Accordingly, Shipp et al or Ritz et al would not have provided any guidance to one of ordinary skill in the art to carry out the invention as claimed. Therefore, Applicants respectfully submit that Shipp et al and Ritz et al fail to anticipate the claimed invention as failing to provide an enabling disclosure which would allow one of ordinary skill in the art to practice the invention as claimed.

Based on the foregoing, Applicants respectfully submit that the claims as currently amended are not anticipated by Shipp et al or Ritz et al. Therefore, Applicants respectfully request that the Examiner withdraw the rejection to claims 1, 2 and 6.

In view of the foregoing, Applicants respectfully submit that the present application is now in condition for immediate allowance, and such action is earnestly solicited.

Respectfully submitted,
LARSON & TAYLOR, PLC



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ATTACHMENT C
Amendments to the Claims

Following herewith is a complete listing of the claims, including a marked copy of the currently amended claims.

C5G

1. (Currently Amended) An isolated polypeptide comprising an amino acid sequence ~~chosen from~~ selected from the group consisting of the sequence SEQ ID ~~No.~~ NO: 2 or SEQ ID ~~No.~~ NO: 4, a sequence derived from or homologous to said sequence SEQ ID ~~No.~~ NO: 2 or SEQ ID ~~No.~~ NO: 4, said derived or homologous sequence having at least 75% sequence identity with SEQ ID NO: 2 or SEQ ID NO: 4, and a biologically active fragment of said sequence SEQ ID ~~No.~~ NO: 2 or SEQ ID ~~No.~~ NO: 4, said isolated polypeptide being referred to as "NEP2 ~~II~~".

2. (Currently Amended) An isolated nucleic acid comprising a nucleotide sequence ~~chosen from the sequence~~ selected from the group consisting of SEQ ID ~~No.~~ NO: 1 or SEQ ID ~~No.~~ NO: 3, a sequence derived from or homologous to said sequence SEQ ID ~~No.~~ NO: 1 or SEQ ID ~~No.~~ NO: 3 having at least 75% sequence identity thereof, and the complementary sequences thereof.

3. (Currently Amended) An oligonucleotide probe ~~which hybridizes specifically with a nucleotide sequence as claimed in claim 2, said probe~~ having a nucleotide sequence chosen from the sequences SEQ ID ~~No.~~ NO: 5 to SEQ ID ~~No.~~ NO: 27.

4. (Original) A cloning and/or expression vector containing a nucleotide sequence as claimed in claim 2.

5. (Original) A host cell transfected with a vector as claimed in claim 4.

C60

6. (Currently Amended) Mono- or polyclonal isolated antibodies or their fragments, chimeric isolated antibodies or immunoconjugates, characterized in that they are obtained using a polypeptide as claimed in claim 1 administered to an animal, and are capable of recognizing specifically a polypeptide as claimed in claim 1.

7-10. (Withdrawn)

C61

11. (Currently Amended) A method for screening compounds which are capable of inhibiting the metalloprotease activity of the NEP2 ~~II~~ polypeptide as claimed in claim 1, ~~in which said compounds are brought into contact with said NEP II polypeptide and the degree of inhibition of the metalloprotease activity of NEP II is evaluated~~; said method comprising the steps of:

measuring NEP2 activity in the presence or absence of a test compound, under conditions sufficient for NEP2 activity to be measured in the absence of a test compound, and

comparing NEP2 activity as measured in the presence of the test compound with that measured in the absence of the test compound,

wherein a decreased activity in the presence of the test compound is indicative of a compound capable of inhibiting the metalloprotease activity.

12. (Withdrawn)

Cl2

13. (Currently Amended) ~~A~~ The method ~~of using the NEP II polypeptide as claimed in~~ according to claim ~~1 for screening~~ 11 further comprising manufacturing a medicinal product from the compounds which are ~~inhibitors of~~ capable of inhibiting the metalloprotease activity of the NEP2 ~~II, and which~~ polypeptide~~g compounds suspected of being capable of inhibiting the metalloprotease activity of the NEP II polypeptide as claimed in claim 1 into contact with said polypeptide and determining which of said compounds inhibit the metalloprotease activity of said NEP II polypeptide~~.

14. (Cancelled)

Cl3

15. (New) The method of claim 13, wherein said medicinal product is useful for treating disorders involving the peptide transmission in which NEP2 participates.

16. (New) The method according to claim 15 wherein said disorder is selected from the group consisting of cardiovascular and neuro-degenerative diseases, growth disorders of endocrine origin, disturbances of the hypothalamo-hypophysial axis and endocrine conditions.

ATTACHMENT A
Substitute Abstract

A Substitute Abstract is provided as follows.

ABSTRACT

An isolated polypeptide is provided which comprises an amino acid sequence selected from the sequences SEQ ID NO: 2 or NO: 4. The sequence is derived from or homologous with the sequence SEQ ID NO: 2 or NO: 4, or a biologically active fragment of the sequence SEQ ID NO: 2 or NO: 4. The isolated polypeptide is designated as NEP2. The NEP2 polypeptide can be used for screening inhibitors useful in therapy.

ATTACHMENT B
Amendments to the Specification

At the following locations, a marked up copy of the replaced paragraph is provided (line numbers provided below correspond to the line numbering provided in the left hand margin of the English translation of the original application).

~~**Page 1, in the title:**~~

C2 "Novel membrane-bound metalloprotease NEP2~~-II~~ and the use thereof for screening inhibitors useful in therapy"

Page 1, lines 5-8:

C3 The subject of the present invention is a novel membrane-bound metalloprotease called NEP2~~-II~~ and the use thereof, in particular for screening inhibitors useful in therapy.

Page 1, lines 9-27:

C4 Membrane-bound metalloproteases such as neprilysin (NEP1~~-I~~, EC 3.4.24.11) play an important role in the activation or inactivation of neuronal or hormonal peptide messengers. Their selective inhibition by synthetic compounds has already led to medicinal products which are commonly used in therapeutics, or which are in the process of clinical development, in particular in the gastroenterological (Baumer et al., Gut, 1992, 33:753-758) and cardiovascular (Gros et al., Proc. Natl. Acad. Sci. USA, 1991, 88:4210-4214) fields. The isolation of the cDNAs of genes of novel related metalloproteases is likely to enable the development of novel classes of specific inhibitors with promising therapeutic uses. It is in this way that the cloning and the

Cont C4

expression of the endothelin-converting enzyme (ECE) gene (Xu et al., Cell, 1994, 78:473-485) allowed the development of inhibitors which are potentially useful in certain cardiovascular disorders.

Page 1, lines 28-32:

C5

The authors of the present invention have revealed a novel membrane-bound metalloprotease belonging to the ECE/NEP/Kell family (Lee S. et al., 1991, PNAS 88(14):6353-57), which they have called NEP2~~-II~~.

Page 1, line 33 through Page 2, line 2:.

C6

A subject of the present invention is thus an isolated polypeptide comprising an amino acid sequence chosen from the sequence SEQ ID~~-No.~~ NO: 2 or SEQ ID~~-No.~~ NO: 4, a sequence derived from or homologous to said sequence SEQ ID~~-No.~~ NO: 2 or SEQ ID~~-No.~~ NO: 4, and a biologically active fragment of said sequence SEQ ID~~-No.~~ NO: 2 or SEQ ID~~-No.~~ NO: 4, said isolated polypeptide being referred to as "NEP2~~-II~~".

Page 2, lines 3-4:

C7

The sequence SEQ ID~~-No.~~ NO: 2 is the amino acid sequence of NEP2~~-II~~ identified in rats.

~~Page 2, lines 5-6:~~

C8

The sequence SEQ ID~~-No.~~ NO: 4 is an amino acid sequence (partial) of NEP2~~-II~~ identified in humans.

Page 2, lines 7-15:

C9

The term "derived" polypeptide is intended to mean any polypeptide resulting from a modification of genetic and/or chemical type of the sequence SEQ ID ~~No.~~ NO: 2 or SEQ ID ~~No.~~ NO: 4, i.e. by mutation, deletion, addition, substitution and/or chemical modification of at least one amino acid, or any isoform having a sequence identical to the sequence SEQ ID ~~No.~~ NO: 2 or SEQ ID ~~No.~~ NO: 4, but containing at least one amino acid in the D form.

Page 2, lines 31-36:

C10

Said homologous polypeptides show preferably greater than 70%, even more preferably greater than 75%, sequence homology with the complete sequence SEQ ID ~~No.~~ NO: 2 or SEQ ID ~~No.~~ NO: 4, the homology being particularly high in that portion of said polypeptide containing the active site.

Page 3, lines 12-18:

C11

Said polypeptides derived from or homologous to, or the polypeptide fragments of, the polypeptide of sequence SEQ ID ~~No.~~ NO: 2 or SEQ ID ~~No.~~ NO: 4 are biologically active, i.e. they have biological properties identical or similar of the biological properties of the NEP2 ~~II~~ polypeptide of sequence SEQ ID ~~No.~~ NO: 2 or SEQ ID ~~No.~~ NO: 4, namely metalloprotease activity.

Page 3, lines 19-26:

C12

The preferred polypeptide fragments comprise the sequence of the active site responsible for binding the zinc atom which is essential for the catalysis. This active site has been identified as encompassing the HEX_1X_2H, H_1 and H_2 residues representing varied amino acids. It is in particular the HEITH sequence (amino acids 608 to 612 of the sequence SEQ ID ~~No.~~ NO: 2) in the NEP2 ~~II~~ polypeptide in rats and humans.

Page 3, lines 27-32:

C13

A subject of the present invention is also an isolated nucleic acid comprising a nucleotide sequence chosen from the sequence SEQ ID ~~No.~~ NO: 1 or SEQ ID ~~No.~~ NO: 3, a sequence derived from or homologous to said sequence SEQ ID ~~No.~~ NO: 1 or SEQ ID ~~No.~~ NO: 3, and the complementary sequences thereof.

Page 3, lines 33-35:

C14

The sequence SEQ ID ~~No.~~ NO: 1 is the cDNA sequence comprising the coding frame for NEP2 ~~II~~ identified in rats.

Page 3, lines 36-38:

C15

The sequence SEQ ID ~~No.~~ NO: 3 is the cDNA sequence comprising (partially) the coding frame for NEP2 ~~II~~ identified in humans.

Page 4, lines 1-10:

C16

The term "derived" nucleotide sequence is intended to mean any nucleotide sequence encoding a polypeptide derived from NEP2~~-II~~ as defined above, i.e. a sequence resulting from a modification of the sequence SEQ ID~~-No.~~ NO: 1 or SEQ ID ~~No.~~ NO: 3, in particular by mutation, deletion, addition or substitution of at least one nucleotide. Included in particular are the sequences which are derived from the sequence SEQ ID~~-No.~~ NO: 1 or SEQ ID~~-No.~~ NO: 3 by degeneracy of the genetic code.

Page 4, lines 11-15:

C17

The term "homologous" sequence is intended to mean more particularly any nucleotide sequence encoding an NEP2~~-II~~ polypeptide homologous to the NEP2~~-II~~ polypeptide of sequence SEQ ID~~-No.~~ NO: 2 or SEQ ID~~-No.~~ NO: 4 in mammalian species other than rats or humans.

Page 4, lines 16-21:

C18

Such a homologous sequence has preferably greater than 70%, even more preferably greater than 75%, homology with the sequence SEQ ID~~-No.~~ NO: 1 or SEQ ID ~~No.~~ NO: 3, the homology being particularly high in the central portion of the sequence encoding the NEP2~~-II~~ polypeptide.

Page 4, lines 22-28:

C19

Preferably, such as homologous nucleotide sequence hybridizes specifically with the sequences which are complementary to the sequence SEQ ID~~-No.~~ NO: 1 or~~-No.~~

Cont C19 NO: 3, under stringent conditions. The parameters which define the stringency conditions depend on the temperature at which 50% of the paired strands separate (Tm).

Page 5, lines 6-10:

C20 The nucleotide sequences according to the invention can be used for producing a recombinant NEP2~~-II~~ protein according to the invention, according to techniques for producing recombinant products, known to persons skilled in the art.

Page 7, lines 1-3:

C21 The preferred probes are in particular the oligonucleotide probes chosen from the sequences SEQ ID ~~No.~~ NO: 5 to SEQ ID ~~No.~~ NO: 27.

Page 7, lines 8-11:

C22 Such probes are also useful in a method for detecting the expression of the NEP2~~-II~~ polypeptide in a cell or tissue sample or in cells or a tissue, by *in situ* hybridization, comprising the steps consisting in:

Page 7, lines 14-19:

C23 - brining said RNA obtained into contact with at least one probe having a nucleotide sequence which is capable of hybridizing specifically with a nucleotide sequence according to the invention, said probe possibly being in particular an oligonucleotide probe of sequence SEQ ID ~~No.~~ NO: 5 to SEQ ID ~~No.~~ NO: 27;

Page 7, lines 20-22:

- detecting the presence of mRNA hybridizing with said probe, which indicates the expression of the NEP2~~-II~~ polypeptide.

Page 7, lines 23-31:

A subject of the invention is also mono- or polyclonal antibodies or their fragments, chimeric antibodies or immunoconjugates, characterized in that they are obtained using a polypeptide according to the invention administered to an animal, and are capable of recognizing specifically a polypeptide according to the invention. A subject of the invention is also the use of these antibodies for purifying or detecting an NEP2~~-II~~ polypeptide in a biological sample.

Page 7, lines 32-36:

The polyclonal antibodies can be obtained from the serum of an animal immunized against the NEP2~~-II~~ protein produced, for example, by genetic recombination using the method described above, according to the usual procedures.

Page 8, lines 7-9:

The antibodies according to the invention are particularly useful for detecting the presence of NEP2~~-II~~.

Page 8, lin s 10-13:

C28 A subject of the present invention is therefore a method for immunologically detecting NEP2~~-II~~ in a cell or tissue sample or in cells or a tissue, comprising the steps consisting in:

Page 8, lines 17-19:

C29 - detecting the presence of said antibody, which is an indication of the presence of the NEP2~~-II~~ polypeptide.

~~**Page 8, lines 26-29:**~~

C30 The antibodies according to the invention can thus make it possible to evaluate overexpression of the ~~[lacuna] II~~ NEP2 polypeptide, which may be an indication of neuroendocrine tumor cells in particular.

Page 8, lines 30-37:

C31 A subject of the invention is also a method for identifying compounds which are substrates for the NEP2~~-II~~ polypeptide as defined above, in which said compounds, optionally labeled, are brought into contact with the NEP2~~-II~~ polypeptide, and the cleavage of said compounds by NEP2~~-II~~, which is an indication of the metalloprotease activity of NEP2~~-II~~ toward said substrate compounds, is evaluated.

Page 8, line 38 through Page 9, line 3:

C32 Such substrates specific for NEP2~~-II~~ can in particular be used in a method for detecting the metalloprotease activity of NEP2~~-II~~ in a cell or tissue sample or in cells or a tissue, comprising the steps consisting in:

Page 9, lines 4-8:

C33 - bringing said cell or tissue sample, said cells or said tissue into contact with a compound which is a substrate for the NEP2~~-II~~ polypeptide, obtained according to the invention, said substrate compound being optionally labeled;

Page 9, lines 9-11:

C34 - evaluating the cleavage of said substrate compound, which is an indication of the metalloprotease activity of NEP2~~-II~~.

Page 9, lines 12-16:

C35 Cells which can be thus assayed are especially cells transfected with a polynucleotide encoding the NEP2~~-II~~ polypeptide as defined above. Tissue extracts which can be assayed are especially testicle membranes, which are particularly rich in NEP2~~-II~~ metalloprotease.

Page 9, lines 17-23:

C36 A subject of the invention is, moreover, a method for screening compounds which are capable of inhibiting the metalloprotease activity of the NEP2~~-II~~ polypeptide

Cont 36

according to the invention, in which said compounds are brought into contact with said NEP2-II polypeptide and the degree of inhibition of the metalloprotease activity of NEP2-II is evaluated.

Page 9, lines 24-26:

C37

The compounds capable of inhibiting the metalloprotease activity of NEP2-II are preferably short peptides of 2 or 3 natural or modified amino acids.

Page 9, lines 27-38:

C38

The synthetic peptides identified as inhibitors of the metalloprotease activity of NEP2-II by this screening method can be coupled to a zinc-chelating group, such as thiol, phosphate or hydroxamic acid groups, according to the conventional techniques known to persons skilled in the art. The inhibitor compound obtained is a good candidate as an active principle of a medicinal product, in combination with a pharmaceutically acceptable vehicle. Said chelating group can optionally be transiently protected, for example with a thiol ester, so as to improve the bioavailability of said active principle.

Page 10, lines 1-7:

C39

The NEP2-II polypeptide according to the invention is particularly useful for screening compounds which are inhibitors of the metalloprotease activity of NEP2-II and which are useful for manufacturing a medicinal product intended for treating disorders involving peptide transmissions in which NEP2-II participates.

Pag 10, lines 16-20:

C40 The compounds which are substrates for NEP2-II or which are inhibitors of the metalloprotease activity of NEP2-II, obtained according to the methods described above, can also be useful for detecting the NEP2-II protein.

Page 10, lines 21-24:

C41 A subject of the present invention is therefore also a method for detecting NEP2-II in a cell or tissue sample or in cells or a tissue, comprising the steps consisting in:

Page 10, lines 25-32:

C42 - bringing said cell or tissue sample, said cells or said tissue into contact with a compound which is a substrate for the NEP2-II polypeptide, obtained as defined above, or with a compound which is an inhibitor of the metalloprotease activity of NEP2-II, obtained according to the screening method as defined above, said substrate compound or said inhibitor compound being labeled;

Page 10, lines 33-35:

C43 - detecting the presence of said substrate compound or of said inhibitor compound, which is an indication of the presence of the NEP2-II polypeptide.

Page 11, lines 7-11:

C44

Cloning the cDNA encoding NEP2-II in rats

Degenerate oligonucleotides were obtained based on the alignment of the peptide sequences of the ECE, NEP1-I and Kell enzymes, and on the delimitation of the regions of strong homology.

Page 11, lines 22-31:

C45

This made it possible to amplify a 420-base pair fragment from the testicle RNAt, encoding an open reading frame which has 76% homology with the NEP1-I protein. This sequence was completed by 3' and 5' RACE (rapid amplification of cDNA ends), using RNAt from brain and from testicles. The sequences were confirmed by verifying five different clones for each tissue and each amplification. The complete cDNA (SEQ ID ~~No.~~ NO: 1) was then cloned into the vectors PCR2.1 and pcDNA3.1 (Invitrogen).

Page 11, line 34 through Page 12, line 14:

C46

Characteristics of the rat NEP2-II polypeptide

The novel gene isolated encodes a 774-amino acid protein (SEQ ID ~~No.~~ NO: 2) which, besides strong homologies with the NEP1-I, ECE and Kell enzymes (52%, 40% and 28% amino acid identity, respectively), has the consensus sequence of the HEXXH active site, a transmembrane region (amino acids 24 to 40 in the sequence SEQ ID ~~No.~~ NO: 2) followed by four cysteine residues which are characteristic of this family, and seven potential glycosylation sites. Three alternative splicings were identified by sequencing the RACE products and by RT-PCR. One of these alternative splicings

Cont C46

eliminates a potential glycosylation site and might affect the transit of the protein to the surface of the cell, or its activity. Each splicing corresponds, moreover, to an exon of NEP1~~-I~~, which suggests a similar gene structure. These data demonstrate that this novel enzyme belongs to the family of ECE/NEP/Kell metalloproteases. Its notable homology with NEP1~~-I~~ led to it being named NEP2~~-II~~.

Page 12, lines 17-25:

C47

Cloning the cDNA encoding NEP2~~-II~~ in humans

In order to clone the human homologue of NEP2~~-II~~, two oligonucleotides were designed, based on the protein sequence of rat NEP2~~-II~~. The sequences were chosen, on the one hand, for their low degeneracy (such as, for example, a tryptophan, represented by a single codon in the genetic code) and, on the other hand, for their degree of conservation (such as the zinc binding site).

Page 12, ~~line 26:~~

C48

1- (H)EITHFD (SEQ ID~~No.~~ NO: 28) or 5' – CGA GAT CAC ACA TGG CTT TGA TGA – 3' (S) (SEQ ID~~No.~~ NO: 22)

Page 12, line 27:

C49

2- QVWCGS (SEQ ID~~No.~~ NO: 29) or 5' – GGA CCC ACA CCA CAC CTG – 3' (AS) (SEQ ID~~-n°~~ NO: 23)

Page 12, lines 28-34:

C50 A polymerase chain reaction was carried out on human hippocampe cDNA obtained from a library (Stratagene), and a 330-bp band was amplified, subcloned and sequenced (SEQ ID ~~No.~~ NO: 3). The sequence obtained shows 82% sequence homology with rat NEP2 ~~II~~, which makes it possible to assert that it encodes the human homologue.

Page 13, line 6:

C51 HNII-1 5' – CGG CCT GGA TCT CAC CCA TGA G – 3' (SEQ ID ~~No.~~ NO: 24)

Page 13, line 7:

C52 HNII-2 5' – CTG ACT GCT CCC GGA AGT GCT GGG TG – 3' (SEQ ID ~~No.~~ NO: 2)

Page 13, line 8:

C53 HNII-3 5' – GAG CAG CTC TTC TTC ATC – 3' (SEQ ID ~~No.~~ NO: 26)

Page 13, line 9:

C54 HNII-4 5' – CTC CAC CAA TCC ATC ATG TTG C – 3' (SEQ ID ~~No.~~ NO: 27)

Page 13, lines 10-17:

NEP2 ~~II~~ tissue expression

C55 Northern blot and RT-PCR studies show that NEP2 ~~II~~ is encoded by a 2.8-Kb transcript which is very highly expressed in rat testicles, and moderately expressed in

the heart, the liver, the digestive system and the brain. Semi quantitative RT-PCR studies show a similar expression profile in these tissues and a predominance of the long forms.

Page 13, lines 23-27:

The native NEP2-II polypeptide is expressed in a heterogeneous manner in the nervous system, the glands (hypophyses, testicle), the digestive apparatus (small intestine in particular) and the cardiovascular system (heart in particular).

Page 13, lines 28-31:

In situ hybridization techniques also indicate a high expression of the NEP2-II protein in neurons and adenohypophysial cells expressing the gene for POMC (propiomelanocortin), which is the precursor of ACTH.

Page 14, lines 1-7:

These locations indicate the participation of NEP2-II in the proteolysis of hormones and of peptide neurotransmitters, or of their precursors, coming from or acting on these diverse organs. It consequently becomes advantageous, for therapeutic purposes, to affect the corresponding peptide transmissions by inhibiting NEP2-II.